were decreased after MI ($p < 0.01$). We observed evidence of increased tropomyosin oxidation by reversible modification of sulfhydryls, and confirmed the oxidized protein's identity using mass spectrometry. These data characterize the relatively unexplored structural and functional modifications to sarcomeres in the early aftermath of MI, and may provide insight into the initial changes that trigger remodeling and heart failure, as well as the contribution of ROS to this process.

**2807-Pos**

**Automated Image Analysis of Electron Micrographs of Structurally Compromised Striated Muscle**

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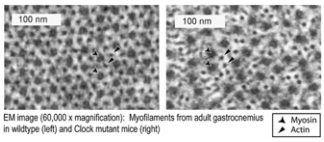
Striated (skeletal and cardiac) muscle is a highly organized and conserved tissue with a molecular structure comprised of bundles of actin (thin filaments) and myosin (thick filaments). We have observed that skeletal muscle from two genetically modified murine models showing disrupted circadian rhythms (Bmal knockout and Clock$^{\Delta 19}$), exhibit significant muscle weakness defined by a reduction in specific tension. Electron micrographs (EMs) of cross-sections from adult gastrocnemius in these mice reveal obvious divergences from the normal hexagonal arrangement of thin filaments around thick filaments.

The goal of this project is to develop a tool for the high-throughput analysis of myofilament architecture. Image processing software written in MATLAB identifies myofilaments in EMs of muscle cross-sections as intensity peaks in the gray-scale image. Filaments are categorized as thick or thin depending on the cross-sectional area of the peaks after thresholding. Structural properties, such as the ratio of thin to thick filaments, the distance to closest neighbors, the angular distribution and the diameter of filaments will be determined for different muscle samples. This quantitative analysis should lead to improved understanding of structure-function relationships in striated muscle.



EM image (60,000 x magnification): Myofilaments from adult gastrocnemius in wildtype (left) and Clock mutant mice (right)

**2808-Pos**

**Myosin-Based Inclusion Body Myopathy Type 3 Decreases Muscle Power Generation and Kinetics**

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Dominant inclusion body myopathy type 3 (IBM-3) results from a point mutation, Glu706Lys, in the SH1 helix of the myosin head in skeletal muscle. The mutation leads to progressive myofibrillar disorganization, rimmed vacuoles and muscle weakness. We are studying *Drosophila* transgenically expressing this myosin mutation in their indirect flight muscle (IFM) and jump muscle. Wing beat frequency (WBF) and jump ability assays were performed on 2-3 day old flies at 25°C. Heterozygous fly WBF was reduced to 123 ± 14 Hz compared to control fly WBF of 181 ± 13 Hz. This decrease contributes to a completely flightless phenotype. Homozygous flies showed no ability to beat wings. A jump ability assay was executed to gauge any changes in myosin function of the *Drosophila* jump muscle, which is similar to very fast vertebrate muscle. No significant impairment of jumping ability was observed in heterozygous mutants, 5.93 ± 0.41 cm compared to control flies, 5.61 ± 1.48 cm. However, homozygous flies were not able to jump. Homozygous skinned IFM fibers at 2-days of age failed to produce power. Mechanical analysis of < 2 hour old skinned heterozygous fly IFM fibers revealed an 85% decrease in maximum oscillatory power generation ($P_{max}$) and an ~6-fold decrease in the frequency at which maximum power was generated ($f_{max}$) compared to controls. We hypothesize that the mutation increases the time myosin spends in a strongly actin bound state, leading to muscle myofibrillar disorganization and decreased power output.

**2809-Pos**

**Titin Isoform Size is not Correlated with Thin Filament Length in Rat Skeletal Muscle**

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The striated muscle sarcomere is dependent upon the precise interactions of a variety of myofibrillar proteins for its proper formation. As the largest and third most abundant protein in this milieu, titin plays a number of functions in the sarcomere, including assembly of the thick filaments and preventing overstretch. The titin gene is expressed as multiple splice variants in skeletal muscle, generating a continuum of titin protein sizes. Recently it was reported that thin filament length was related to titin size, and that the latter might be involved in determining thin filament length. We tested this hypothesis using several muscles from wild type rats and from a mutant rat model (Greaser et al J Mol Cellul Cardiol 44:983, 2008) which results in increased titin size. Myofibrils were isolated from skeletal muscles (diaphragm, extensor digitorum longus, gastrocnemius, longissimus dorsi, psoas major, rectus abdominis, and tibialis anterior) using both adult wild type (WT) and homozygous mutant (HM) rats (n=6 each). Thin filament length was estimated using fluorescent dye labeled phalloidin and relaxed sarcomere length was determined by phase contrast microscopy after adding ATP and BDM. No differences in thin filament lengths were found between WT muscles with titin sizes ranging from 3.2 to 3.7 MDa. Similarly the thin filament lengths in the mutant rats did not differ from the paired WT muscles in spite of large differences in titin size with several muscles. However, the relaxed sarcomere length was correlated to titin size in muscles from WT rats, and it was significantly increased relative to WT for within muscle comparisons. The data indicates that, although titin performs many functions, its relationship to thin filament length could not be demonstrated in the rat. Supported by HL77196.

**2810-Pos**

**Conformation of A-Band Titin**

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The giant protein titin has important roles in the assembly, signalling and passive mechanical properties of muscle sarcomeres. Titin is formed by a single polypeptide with isoforms ranging between 3 and 4 MDa. This folds into ~300 immunoglobulin (Ig) and fibronectin (Fn3) domains in a beads-on-a-string-like chain more than 1 μm long. The N-terminal half of the molecule forms an elastic connection between the end of the thick filament and the Z-line. The C-terminal half is bound to the thick (myosin) filament. Through most of the thick filament region, the Ig and Fn3 domains are arranged in a distinctive eleven domain 'large super-repeat', Ig-Fn-Fn-Ig-Fn-Fn-Fn-Ig-Fn-Fn-Fn. Eleven copies of the large super-repeat make up ~0.5 μm of the titin molecule length. In an attempt to reconstruct the structure of this region, we have studied a set of two- and three-domain recombinant fragments forming a large super-repeat using electron microscopy, synchrotron X-ray solution scattering and analytical ultracentrifugation. The data illustrate different average conformations in different domain pairs, correlating with differences in lengths of the inter-domain linkers. They also illustrate a level of flexibility between domains in all pairs around average states. Overall, the results suggest the large super-repeat forms an irregular helix, and is also likely to be dimerized *in situ*.

**2811-Pos**

**Effect of Excision of Titin's PEVK Exons 219-225 on Skeletal Muscle Structure and Function**

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We recently published a PEVK KO mouse model in which all exons that constitute the PEVK region of cardiac titin (N2B cardiac isoform), exons 219-225, had been excised, and reported cardiac hypertrophy and increased passive stress (Granzier et al., 2009 *Circ Res*., 11, 557). Here we investigated the phenotype of EDL (fast twitch) and soleus (slow twitch) skeletal muscle of wildtype and homozygous PEVK KO mice. Muscle mass was significantly increased in both soleus (51 ± 5%) and EDL (21 ± 6%) muscles; we are currently studying whether the underlying hypertrophy mechanisms are similar to those previously found in the heart. Because the excised exons make up a small portion of the PEVK segment of skeletal muscle titins we expected modest differences, if any at all, in passive stress. Unexpectedly, passive stress was significantly increased in soleus and EDL muscles, both when measurements (SL 3.0μm) were made at the whole muscle level (40 ± 7% and 67 ± 16%, respectively) and at the fiber bundle level (50 ± 14% and 81 ± 1%). Gel electrophoresis revealed, in both EDL and soleus, expression of a single titin isoform in wildtype muscle, but surprisingly, co-expression of two isoforms in the KO muscles. The larger isoform co-migrated with the isoform expressed in wt muscle and thus is likely to